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Toxicity to amphibians of environmental extracts from natural waters in National Parks and Fish and Wildlife Refuges

Christine M. BRIDGES & Edward E. LITTLE

US Geological Survey, Columbia Environmental Research Center,
4200 New Haven Road, Columbia, Missouri 65201, USA

Amphibian population declines are not limited to overtly degraded habitats, but often occur in relatively pristine environments such as national parks or wildlife refuges, thus forcing biologists to examine less obvious causes for declines such as the presence of contaminants. The objective of our study was to extract naturally-occurring compounds from amphibian habitats (using semipermeable membrane devices) in three national parks or wildlife refuges (two sites within Sequoia Kings Canyon National Park, Big Bend National Park, and Kenai National Wildlife Refuge), and assess their toxicity to developing larvae using bioassays. Extracts did not cause mortality, so all effects observed were sublethal, influencing life history characteristics. In all three areas studied, amphibians reared in extracts from at least one of the two sites exhibited either a lengthened larval period or reduced mass at metamorphosis. Extracts from both the air and water at one site in Sequoia Kings Canyon National Park lengthened the larval period, which is in agreement with studies showing elevated levels of aerially transported contaminants at sites such as this within the park. Ultraviolet radiation, which is also suspected of having caused amphibian declines and was included as a factor in our study, did not act alone or alter the toxicity of the extracts.

INTRODUCTION

Reports of amphibian population declines in seemingly pristine environments, particularly in the western United States, have become increasingly common (CAREY, 1993; FELLERS & DROST, 1993, BRADFORD et al., 1994; CORN, 1994, JENNINGS & HAYES, 1994, KNAPP & MATTHEWS, 2000). Although in some areas of the west the status of amphibian populations remain largely unstudied (e.g., along the Rio Grande river, JUNG et al., 2002), declines have been documented throughout the Sierra Nevada mountains (BRADFORD et al., 1993), in western Colorado (CAREY, 1993) and in the Cascade mountains (MCALLISTER et al., 1993), among other locations. Although few declines have been definitively linked with causes, others cases have been more clear (KNAPP & MATTHEWS, 2000).

Environmental contamination has been proffered as having contributed to some of these declines. For instance, DAVIDSON et al (2001, 2002) found a strong positive relationship between observed patterns of declines in a number of species in the Sierra Nevada mountains and upwind agricultural land usage in the Central Valley of California. SPARLING et al. (2001) correlated a reduction in cholinesterase levels (which may indicate exposure to certain types of toxicants) with areas of population decline in the Sierra Nevadas. It is often difficult, however, to determine what specific contaminants are present at a site or in what concentrations, which often hinders determining the influence of exposure to contaminants on the populations of organisms inhabiting the area. Furthermore, several environmental variables (e.g., pH, Dissolved Oxygen Content, predators) may be acting alone or in combination with the contaminants, contaminants may be present in complex mixtures, and/or the identity and concentrations of specific substances and their metabolites within the composition of such mixtures may be unknown or difficult to determine.

One of our goals was to expose developing amphibian larvae to composite samples of waterborne contaminants in various sites within national parks and fish and wildlife refuges, using in-laboratory bioassays. To accomplish this, we deployed semipermeable membrane devices. These are integrative samplers containing lipid that accumulates lipophilic organic compounds from the environment in a manner that is similar to aquatic organisms (HUCKINS et al., 2002). The uptake rates of fat-soluble compounds by semipermeable membrane devices can be used to define the approximate daily exposure to lipophilic compounds by aquatic organisms (HUCKINS et al., 2002). Thus, the composite waterborne environmental contaminant exposure of organisms to the bioavailable lipophilic compounds present at the site can be accomplished by using extracts from semipermeable membrane devices as toxicant solutions for bioassays (PETTY et al., 2000; HUCKINS et al., 2002) based on the duration of deployment in the environment. Using semipermeable membrane device extracts in bioassays has been shown to be an effective way to assess the toxicity and teratogenicity of naturally occurring environmental compounds to larval anurans (BRIDGES & LITTLE, 2003; BRIDGES et al., 2004).

Ultraviolet (UV) radiation has also been suspected of causing declines among amphibian populations. Exposure to UVB radiation at larval and egg stages has been suspected of negatively impacting some amphibian species directly by reducing hatching success and increasing rates of embryonic malformation (BLAUSTEIN et al., 1998, LIZANA & PEDRAZA, 1998, BROOMHALL et al., 2000, STARNES et al., 2000) as well as generating larval malformations (ANKLEY et al., 2000). And although being a lesser factor than contaminants, DAVIDSON et al. (2002) suspected UV as a likely contributor to amphibian declines in the Sierra Nevada mountains. However, because no environmental factor acts in isolation, it has become clear that single-factor explanations simply may not be sufficient to explain this widespread phenomenon of population declines. For example, UV radiation is also known to increase the toxicity and/or teratogenicity to amphibians of various compounds in aquatic habitats (ZAGA et al., 1998; LITTLE et al., 2000; BRIDGES et al., 2004).

One of our objectives was to investigate whether exposure to lipophilic compounds extracted from natural environments located in conventionally pristine areas (i.e., Sequoia Kings Canyon National Park, Big Bend National Park, Kenai National Wildlife Refuge) can negatively affect developing amphibian larvae. Furthermore, because UV radiation can also

impact amphibians (in and of itself as well as when in combination with environmental contaminants), the effects of UV radiation were also explored. In this study we examined the effects of these two factors, and their interaction, on amphibian larval survival, length of the larval period, and size at metamorphosis for various anuran species.

Materials and methods

Semipermeable membrane device exposure and extraction

To determine whether contaminants present at our study sites can cause mortality or malformations in larval amphibians, we deployed semipermeable membrane devices at two sites within each refuge or national park. Five standard semipermeable membrane devices (described in Huckins et al., 2002) were placed in each of three replicate stainless-steel canisters attached to a steel cable anchored to the shore. The conditions of each deployment are given below.

At the time of deployment a metal can containing semipermeable membrane devices (hereafter, field blank) was opened and exposed to the air at each site. These provided a control for any airborne contaminants present while the semipermeable membrane devices were exposed to the air (i.e., before being placed into the water). Once the semipermeable membrane devices were in the water, the cans containing the field blanks were sealed and stored at 0-4°C for the time between semipermeable membrane device deployment and retrieval to halt contaminant uptake (from the site air that had filled the can). During retrieval, field blanks were once again exposed to the air at the site for as long as it took to remove the semipermeable membrane devices from the water and seal them in cans. After retrieval, both semipermeable membrane devices and field blanks were shipped on ice and remained frozen until they were processed at the Columbia Environmental Research Center (Columbia, Missouri) using techniques outlined in Petty et al. (2000).

All semipermeable membrane device extracts from each site were pooled into a single composite sample. Field exposure and control extracts (i.e., field blanks) were dissolved into sterile dimethylsulfoxide (DMSO) by solvent exchange. Semipermeable membrane device extracts were added to 90 ml of sterile DMSO so that each ml contained the approximate equivalent of a 1-d exposure (i.e., representing the amount of bioavailable residues extracted from site water by a standard semipermeable membrane devices in one days' time) when added to 1 l of water.

Assuming lentic conditions, an ambient temperature between 10 and 18° C, and minimal biofouling, 1-gram equivalent of a standard 1-ml triolein semipermeable membrane devices will clear or extract hydrophobic organic contaminants (e.g., PAH, PCB, organochlorines, pyrethroids) from about 0.01 to 2.0 l/g of water daily (Huckins et al., 2002). Although much greater variability exists in the uptake rate constants of aquatic organisms for the same chemicals, the values for invertebrates and fishes generally range from 0.03 to 8.0 l.d^{-1}.g^{-1} (Mackay et al., 1991, 1992, 1997). Thus, it is reasonable to expect that aliquots of semipermeable membrane device extracts, which represent the daily volume of water extracted by a

whole standard semipermeable membrane devices, are representative of the amounts of chemicals to which aquatic organisms (e g., tadpoles) are exposed daily.

COLLECTION SITES AND CONDITIONS

Because our semipermeable membrane devices were deployed during various times of the year, the extracts may not be composed of the same compounds that might be available during the spring, when many amphibian species breed. However, semipermeable membrane devices are designed to sample very persistent contaminants (PETTY et al , 2000), and whereas the concentrations of the waterborne lipophilic contaminants may change with increased runoff, etc , the composition of these contaminants would likely change very little. Consequently, sampling the water in the late summer/early fall likely represents a conservative effects assessment. Regardless of when our semipermeable membrane devices were deployed, the time of deployment coincides with at least partial development of most species in these habitats.

We attempted to choose sites within each park or national refuge that were disparate in their habitats (e g , one high and one low elevation), initially wishing to select one site that would be more contaminated than the other. Species used within two of the three tests are species found within our sampling area. In one case (i.e , Alaskan sites), however, this was not possible and we used a surrogate species.

Sequoia and Kings Canyon National Park

The first site within Sequoia and Kings Canyon National Park was at Yucca Creek, just east of the western border of the park It is a mostly shaded, slowly flowing, clear stream with a rocky bottom. Semipermeable membrane devices were deployed at approximately 1 m underwater Water temperature was 16°C when the devices were deployed on 12 September 2000 and when they were retrieved on 11 October 2000 Upon retrieval there was no evidence that the devices had been disturbed

The second site within Sequoia and Kings Canyon National Park was in Aster Lake, a clear, lentic alpine lake at about 2800 m elevation approximately 8 km west of the Wolverton trail head within the park The site is not shaded and the semipermeable membrane devices rested 1 5 m underwater The water temperature was 10°C when the devices were deployed on 13 September 2000 and 12°C upon retrieval on 12 October 2000. Upon retrieval there was no evidence that the devices had been disturbed.

Big Bend National Park

The first site within Big Bend National Park was in the Rio Grande River approximately three miles from Castalon The water, which was unshaded, muddy and had a moderate flow, was 23°C upon deployment of the devices on 4 April 2001 We are uncertain as to the exact depth of deployment but anticipate it was at least 2 m Upon deployment, the amount of UVA transmitted through the water column at a depth of 10 cm was 9,700 uW/cm^2 and the UVB was 10 3 uW/cm^2 when measured with a Macam Photometrics broadband UV meter Upon

retrieval on 10 May 2001 the water temperature was 24°C and the devices had a substantial amount of mud and grime (i e , biofouling) coating them, but there was no evidence of them having been disturbed.

The second site within Big Bend National Park was at Lower Cattail Falls, at the end of a trailhead, the entrance of which is across the street from the Sam Nail Ranch. This site is a shaded clear flowing stream and the device was anchored in 1 m of water. The water temperature was 19 8°C when the devices were deployed on 5 April 2001 and was 21°C when the devices were retrieved on 10 May 2001. Upon deployment, the amount of UVA transmitted through the water column at a depth of 10 cm was 1,182 uW/cm^2 and the UVB was 40.4 uW/cm^2 when measured with a Macam Photometrics broadband UV meter There was no evidence of biofouling or disturbance.

Kenai National Wildlife Refuge

Both sites within the Kenai National Wildlife Refuge are located within the Swanson River unit Both are boggy, unshaded wetlands adjacent to oil fields. All devices were deployed on 11 July 2001 and retrieved on 8 August 2001. At Oil Field 1 (Parsons, 2001) the water temperature was 16°C when the devices were deployed and 21°C when they were retrieved. Upon deployment, the amount of UVA transmitted through the water column at a depth of 10 cm was 2,190 uW/cm^2 and the UVB was 22.6 uW/cm^2 when measured with a Macam Photometrics broadband UV meter. At our second site, Oil Field 3 (Parsons, 2001), the water temperature was 18°C at deployment and 19°C at retrieval. Upon deployment, the amount of UVA transmitted through the water column at a depth of 10 cm was 2,940 uW/cm^2 and the UVB was 46.2 uW/cm^2 When each set of devices was collected, no biofouling had occurred and there was no evidence of tampering

Exposure to semipermeable membrane device extracts

Exposures were carried out over two years due to space and time limitations. In June 2001, Pacific treefrog (*Pseudacris regilla*) tadpoles from three egg masses were received from Sunriver, Oregon to be used in tests with extracts from Sequoia and Kings Canyon National Park. The exposure began on 8 June 2001 and was completed 40 d later when the last tadpole reached metamorphosis. In April 2002, three spring peeper (*Pseudacris crucifer*) egg masses were collected from a farm pond in Boone County, Missouri to use in the tests of extracts from Kenai National Wildlife Refuge. This exposure began on 20 April 2002 and ended 51 d later when the last tadpole reached metamorphosis. We selected the spring peeper as a surrogate test species for the native wood frog because of its availability from an uncontaminated habitat, its comparatively short developmental time to metamorphosis, and its high sensitivity to environmental contaminants. Birge et al. (2000) ranked the spring peeper as highly sensitive based upon acute toxicity tests of 34 inorganic elements and 27 organic chemicals. In these tests the spring peepers consistently exhibited lower tolerance for such exposures than any of the ranid species tested. Thus, any results obtained using this species should yield a conservative estimate of effects that wood frogs would experience In June 2002, canyon treefrog (*Hyla arenicolor*) tadpoles were collected from Big Bend National Park to be

used in exposures to extracts from that park. The exposure began on 21 June 2002 and was completed 45 d later when the final tadpole reached metamorphosis.

The experiment was designed as a 2 × 5 complete factorial: tadpoles were exposed to one of two UV light treatments and to one of five semipermeable membrane device treatments (see below), replicated three times.

Test solutions were created by filling 3-l beakers with 2 l of well water, and adding 1.0 ml of the appropriate semipermeable membrane device extract treatment (hereafter, semipermeable membrane devices treatment) or field blank semipermeable membrane devices. We also used a well water (pH 7.8; hardness 286 mg/l $CaCO_3$; alkalinity 258 mg/l $CaCO_3$) control.

Our high UV treatment (16.2 $\mu W/cm^2$ UVB) was achieved by wrapping the sides of each chamber in polycarbonate plastic (0.030 inch thickness, Cope Plastics, Inc., St. Louis, Missouri) and covering the top of the chamber with two pieces of cellulose acetate (0.015 inch thickness, Cope Plastics, Inc., St. Louis, Missouri) and one piece of and shade cloth (50 % shading, Lowe's Home Improvement Center). The low UV treatment (<1 $\mu W/cm^2$ UVB) was created by wrapping the sides of the chambers with Mylar D (0.005 inch thickness, Cope Plastics, Inc., St. Louis, Missouri) and covering the tops of the chambers with two pieces of polycarbonate plastic and one piece of shade cloth.

Prior to testing, the irradiance level of each treatment was measured with a spectroradiometer at a 10-cm depth in a solar-simulating chamber, as described in LITTLE & FABACHER (1996). The high irradiance value we used throughout the experiment in the solar simulator fell below the mean value we measured in the field at a 10 cm depth. This corresponds to the depth at which many egg masses are found and where they can be found thermoregulating and feeding. The simulator was programmed on a 16L:8D photoperiod. UVB lights were activated five hours into the light cycle for five hours to simulate midday solar intensity. This lighting regime provides for the induction of cellular photorepair functions and prevents the over-estimation of UV-induced injuries.

Groups of three tadpoles were placed in the 3-l jars randomly arranged under a solar simulator in a 20°C flow-through water bath. Tadpoles were exposed to one of five semipermeable membrane device treatments: (1) a well water control, (2) semipermeable membrane device extract from the first site in the refuge/national park; (3) field blank extract from the first site; (4) semipermeable membrane device extract from the second site within the refuge/national park; and (5) field blank extract from the second site. Each time water was changed (i.e., every third day), 1 ml of the appropriate extract (the equivalent to a 2-d dose) was added to the jars. Tadpoles were fed ground fish flakes (Tetra-Min® brand) ad libitum at each test water change until metamorphosis, which was defined as the emergence of at least one forelimb (stage 42, GOSNER, 1960). At metamorphosis individuals were removed from the experimental chamber and housed in the laboratory until tail resorption (about 4 d, or stage 46, GOSNER,1960), when they were weighed to the nearest 0.1 mg.

STATISTICAL ANALYSES

Values for the three tadpoles in each replicate chamber were pooled to attain a single data point. For each location we used analysis of variance (ANOVA) to determine whether mass at

metamorphosis and the length of the larval period were dependent on semipermeable membrane device extract treatment, UV treatment, or the interaction of these factors. Both endpoints were log-transformed to increase normality In analyses of mass at metamorphosis, length of the larval period was used as a covariate and vice versa because these two variables can be correlated. In the instances where the type I sums of squares of these covariates were not significant, they were removed from the model. When there were significant differences among semipermeable membrane device extract treatments, Bonferroni multiple comparison tests were conducted to discern differences among specific treatments at $\alpha = 0.05$ Differences in survival were minimal (i.e., nearly all animals survived through metamorphosis), so no analyses were conducted on this endpoint.

Results

In Sequoia and Kings Canyon National Park, the effect of extract treatment on the size at metamorphosis of tadpoles was marginally significant (tab. 1). Individuals reared in the water controls were significantly larger at metamorphosis than tadpoles in extracts from Yucca Creek (fig 1). However, Yucca Creek tadpoles were not significantly smaller than tadpoles reared in extracts from Aster Lake, or in either of the field blank treatments. The effect of extract treatment on the length of the larval period was not significant However, when the effects of UV (which were not significant) were removed from the model, the main effect of semipermeable membrane device extract treatment became marginally significant ($F_{4,24} = 2,58$, $P = 0.0634$). Tadpoles reared in SPMD extracts from Aster Lake and in extracts from the Aster Lake field blank took longer to reach metamorphosis than those from any other treatment (fig. 2). Neither UV treatment alone nor the interaction between the UV treatment and the semipermeable membrane device extract treatment significantly affected the length of the larval period (tab. 1) or the mass at metamorphosis.

In Big Bend National Park, semipermeable membrane device extract treatment significantly affected the number of days it took for tadpoles to reach metamorphosis (tab. 1) Individuals reared in water containing extracts from the Rio Grande River took significantly longer to transform than individuals reared in field blanks from either site, or controls (fig 3) The length of the larval period in tadpoles reared in water from Lower Cattail Falls was not significantly different than those from the Rio Grande River, and did not differ from controls or field blanks from either site (fig. 3).

At Kenai National Wildlife Refuge, semipermeable membrane device extract treatment significantly affected the length of the larval period (tab. 1) Individuals reared in semipermeable membrane device extracts from Oil Field 3 took significantly longer to reach metamorphosis than individuals reared with Oil Field 3 field blanks, Oil Field 1 semipermeable membrane device extracts, or the control (fig 4). The length of the larval period was not significantly affected by UV treatment or the interaction between semipermeable membrane device treatment and UV Semipermeable membrane device extracts from Kenai National Wildlife Refuge also significantly affected the size at metamorphosis for tadpoles. Individuals reared in extracts from Oil Field 3 were significantly smaller than those reared in any other

Table 1. – Analyses of variance on the effects of SPMD extract (extract), UV radiation (UV) and their interaction on the length of the larval period (days) and the mass at time to metamorphosis (mass) for amphibian larvae. Type III mean squares (*MS*) are reported BBNP, Big Bend National Park; KNWR, Kenai National Wildlife Refuge; SEKI, Sequoia Kings Canyon National Park.

Response variable	Source	*df*	*MS*	*F*	*P*
Days (SEKI)	Extract	4	0 0053	1.80	0.1705
	UV	1	0 0013	0 47	0 5032
	Extract × UV	4	0 0010	0.35	0 8403
	Error	19	0 0029		
Mass (SEKI)	Extract	4	0 0509	2.67	0 0637
	UV	1	0 0174	0.92	0.3505
	Extract × UV	4	0 0086	0.45	0.7690
	Error	19	0.0190		
Days (BBNP)	Mass	1	0.0058	2.02	0.1718
	Extract	4	0 0122	4.25	0 0126
	UV	1	0 0022	0.76	0 3930
	Extract × UV	4	0 0014	0.48	0 7481
	Error	19	0.0029		
Mass (BBNP)	Days	1	0 0295	1.72	0 2053
	Extract	4	0.0217	1.57	0 2223
	UV	1	0.0035	0.21	0 6549
	Extract × UV	4	0.0019	0.12	0 9751
	Error	19	0.0172		
Days (KNWR)	Mass	1	32.39	6 15	0.0227
	Extract	4	15.61	2.96	0.0464
	UV	1	1 19	0.23	0 6394
	Extract × UV	4	7.99	1.52	0 2371
	Error	19	5.26		
Mass (KNWR)	Days	1	0 08	6.31	0 0212
	Extract	4	0.05	3.72	0.0212
	UV	1	0.00	0.07	0.7887
	Extract × UV	4	0 03	2.25	0.1020
	Error	19	0 01		

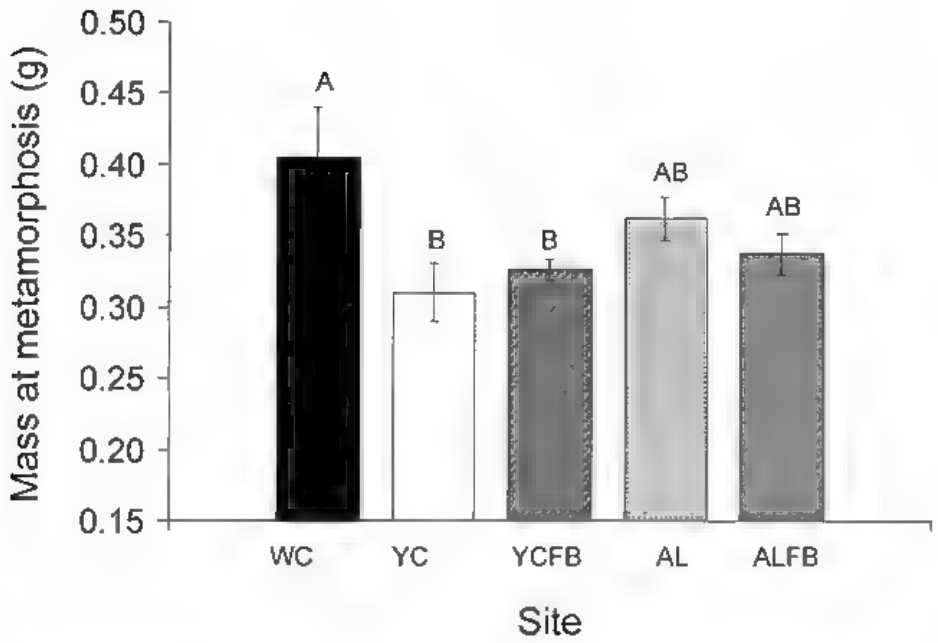

Fig 1 - Mass at metamorphosis for *Pseudacris regilla* tadpoles reared in each treatment for Sequoia Kings Canyon National Park AL, Aster Lake, ALFB, Aster Lake Field Blank, WC, water control; YC, Yucca Creek; YCFB, Yucca Creek Field Blank. Vertical lines represent ± 1 s_x.

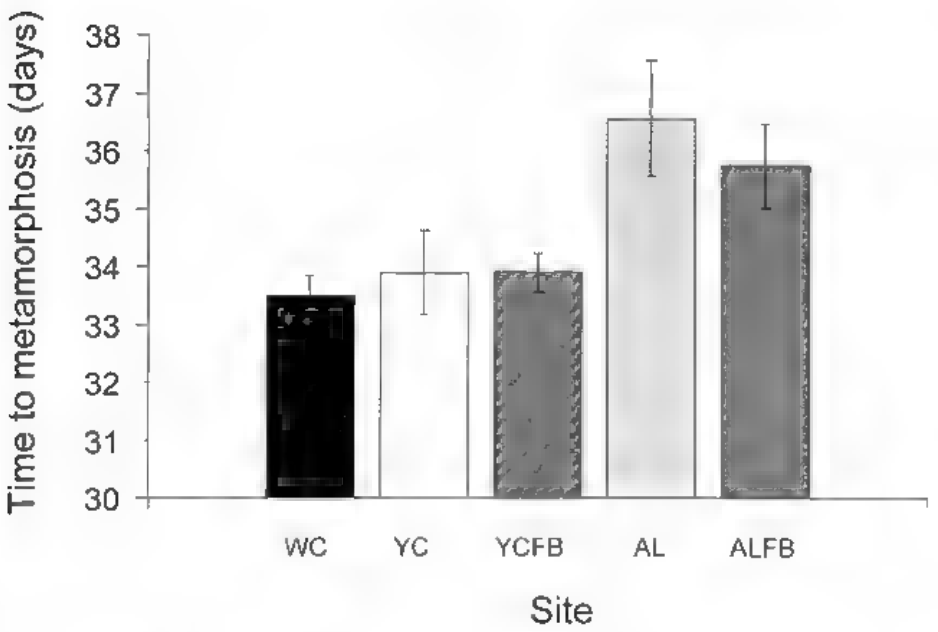

Fig 2 Time to metamorphosis in days for *Pseudacris regilla* tadpoles reared in each treatment for Sequoia Kings Canyon National Park AL, Aster Lake, ALFB, Aster Lake Field Blank, WC, water control, YC, Yucca Creek, YCFB, Yucca Creek Field Blank Bars with the same letters do not differ significantly from one another. Vertical lines represent ± 1 s_x

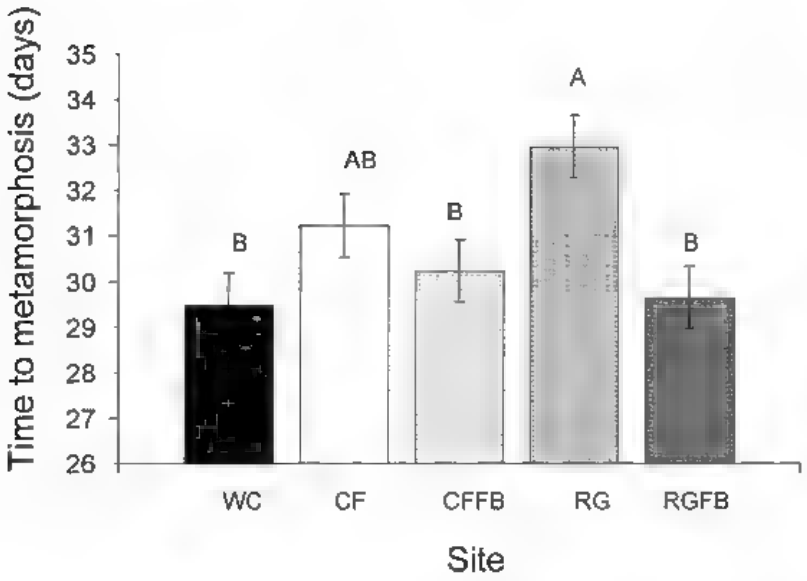

Fig 3 Time to metamorphosis in days for *Hyla arenicolor* tadpoles reared in each treatment for Big Bend National Park. CF, Cattail Falls, CFFB, Cattail Falls Field Blank, RG, Rio Grande; RGFB, Rio Grande Field Blank; WC, water control. Bars with the same letters do not differ significantly from one another. Vertical lines represent $\pm$ 1 $s_{\bar{x}}$.

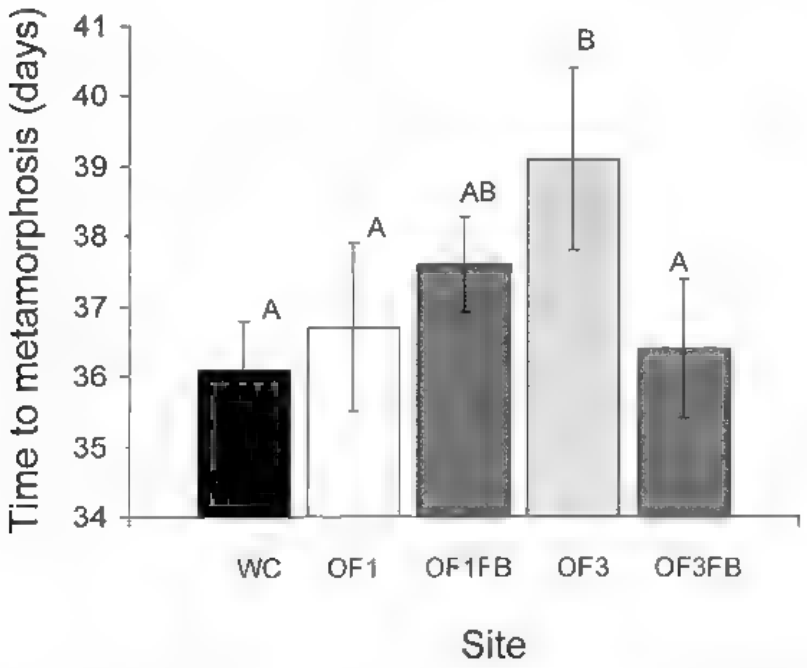

Fig 4 Time to metamorphosis in days for *Hyla arenicolor* tadpoles reared in each treatment for Kenai National Wildlife Refuge. OF1, Oil Field 1, OF1FB, Oil Field 1 Field Blank, OF3, Oil Field 3, OF3FB, Oil Field 3 Field Blank, WC, water control. Bars with the same letters do not differ significantly from one another. Vertical lines represent $\pm$ 1 $s_{\bar{x}}$.

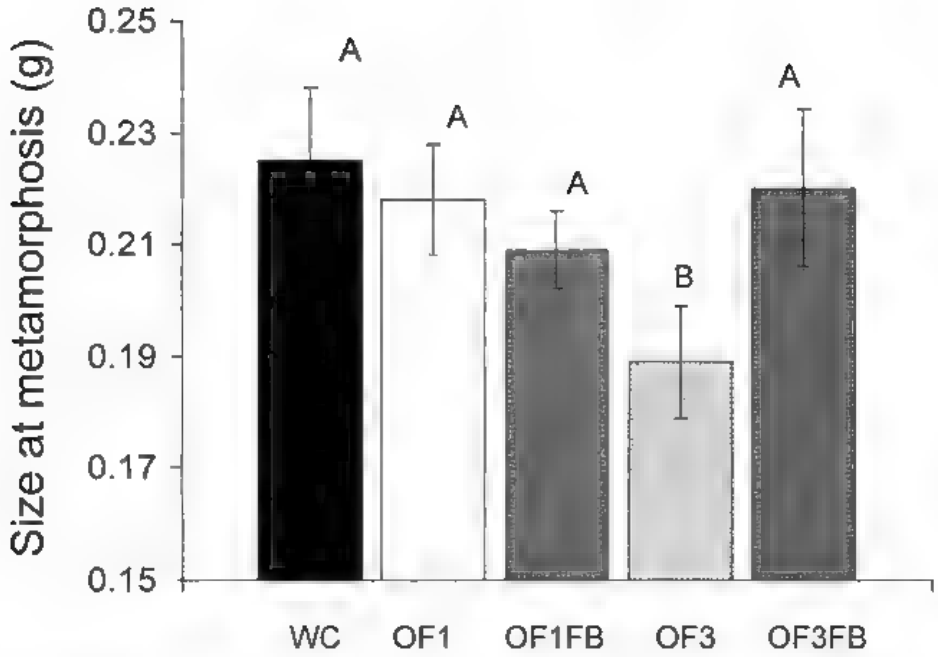

Fig. 5 Mass at metamorphosis in days for *Pseudacris crucifer* tadpoles reared in each treatment for Kenai National Wildlife Refuge. OF001, Oil Field 1, OF001FB, Oil Field 1 Field Blank, OF003, Oil Field 3; OF003FB, Oil Field 3 Field Blank, WC, water control. Bars with the same letters do not differ significantly from one another. Vertical lines represent $\pm$ 1 $s_{\bar{x}}$.

treatment (fig. 4). There was no effect of UV radiation or the interaction between UV radiation and semipermeable membrane device extract treatment on the size at metamorphosis.

Discussion

Utilizing semipermeable membrane device extracts in amphibian bioassays has been shown to be an effective tool in determining whether lipophilic compounds found in aquatic amphibian habitats are toxic (Bridges et al., 2004) without actually having to determine which compounds are present. Importantly, using semipermeable membrane devices rules out detrimental effects attributable to parasites or pathogens because the pore size of the membrane is too small (i.e., 10 Å) to allow passage of bacterial, viral or fungal cells. Although we did not attempt to determine which compounds were present in the extracts used in our experiments, once the presence of contaminants in the environment are confirmed in a bioassay it would be possible to resample the sites and generate a contaminant profile.

Despite the fact that Sequoia and Kings Canyon National Park is a protected, natural area, the presence of contaminants has been revealed throughout the park. Surveys of the

Sierra Nevada mountains around Sequoia and Kings Canyon National Park indicate the presence of chlorpyrifos, malathion, diazanon and DDT in water (ZABIK et al., 1993; MCCONNELL et al., 1998) and in frog tissue (CORY et al., 1970) even at high altitudes within the park. SPARLING et al. (2001) revealed reduced cholinesterase levels of native amphibians in Sequoia Kings Canyon National Park, suggesting the presence of cholinesterase-inhibiting pesticides. DAVIDSON et al (2001, 2002) correlated the amount of downwind pesticide use in California's Central Valley with declines in amphibian populations within the park LENOIR et al (1999) have shown the presence of diazanon and chlorpyrifos in atmospheric transport at elevations where native amphibians are showing the greatest declines.

Our data using Pacific treefrogs (*P. regilla*, which are native to the Sequoia and Kings Canyon National Park region) suggests the presence of a compound in the water at Yucca Creek that generates a smaller size at metamorphosis, which can have a number of fitness consequences (ALTWEGG & REYER, 2003) Individuals that are large at metamorphosis can realize higher overwintering success, greater survival to first reproduction, and earlier reproduction (SMITH, 1987; SEMLITSCH et al., 1988). Further, larger females can carry more eggs, and larger males often gain access to a greater number of females during breeding, leading to increased reproductive success (BERVEN, 1982). The fact that there were no significant differences when comparing Yucca Creek with the other treatments may suggest the presence of a compound in all of our semipermeable membrane device extracts and field blanks that depresses growth (although not detectable significantly in the other treatments).

There was a trend for frogs reared in extracts from Aster Lake and the Aster Lake field blank to take longer to reach metamorphosis. This effect was marginally significant only after the removal of UV effects, perhaps because our statistical power was low. That reductions were observed both in the field blank and the waters extracts from this high elevation site suggests the presence of a compound that is airborne and/or waterborne. This is in agreement with data that indicate the presence of contaminant compounds at high elevations in the Sierra Nevadas suspected of causing declines (LENOIR et al., 1999). Frogs delaying metamorphosis can suffer some of the same fitness detriments as animals undergoing metamorphosis at a small size.

In 2002, Big Bend National Park was designated an endangered park primarily due to its increasingly high levels of human impacts over the past 15 years. Poor air quality attributable to drift from coal-fired power plants is common Increasing aquatic pollution and water diversion upstream has caused the water quality to decline in the Rio Grande River, which is the park's most prominent source of water In fact, low water levels lead to concentration of aquatic pollutant from upstream municipal and agricultural sources in the US and Mexico, further worsening the quality of aquatic habitat in this river.

Metamorphosis was delayed among canyon treefrog (*H. arenicolor*) tadpoles, which are native to Big Bend National Park, developing in water containing extracts from the Rio Grande River Levels of lipophilic contaminants (including DDT) have been detected at sampling sites upstream and downstream of the park (SCHMITT et al., 2004) and in biota feeding in this stretch of the river (MORA et al., 2002). This suggests that amphibian larvae developing in the Rio Grande, or in pools formed by its floodwaters, are at a potential disadvantage which may result in decreased fitness. Tadpoles reared in extracts from Cattail Falls did not take significantly longer to develop than tadpoles in the Rio Grande, but also did

not differ from the controls. No effects were seen due to extracts from the field blanks, indicating that the contaminants sampled at each site were waterborne.

The Kenai National Wildlife Refuge covers nearly 2 million square acres on Alaska's Kenai Peninsula, south of Anchorage. In recent years, activities have taken place on the Kenai National Wildlife Refuge that have increased chemical contamination on this site including oil and gas development, pesticide application, military and recreational uses, mining, and use of fire retardants (Parsons, 1991). Many of these sites were impacted with little or no post-contamination remediation efforts undertaken, primarily because of Alaska's remoteness. We selected two ponds on the Kenai National Wildlife Refuge adjacent to oil drilling operations and that also contain populations of wood frogs (*Rana sylvatica*).

At Kenai National Wildlife Refuge, frogs exposed to semipermeable membrane device extract from the pond at Oil Field 3 were smaller at metamorphosis and took longer to reach metamorphosis than frogs exposed to any other treatment. Why there were effects at Oil Field 3 and not Oil Field 1, which are both adjacent to oil drilling operations, is unclear. The fact that the growth and development of the frogs reared in extract from the Oil Field 3 field blank did not differ from the water control indicates that the contaminant in question is aquatic and not airborne. Whereas a number of petroleum hydrocarbon spills have been documented in the Swanson River Oil Field (Parsons, 2001), where our two sites were situated, it is unclear whether any of these historic spills occurred near our study sites. Without chemical analysis of the semipermeable membrane device extracts, it is not known what chemicals may have elicited this biological response, nor whether this phenomenon is related to oil field operations. Hydrocarbons, among other chemicals, have been shown to be made more toxic in the presence of UV radiation (Zaga et al., 1998, Little et al., 2000). Because there was not a significant interaction between the semipermeable membrane device extracts and UV radiation, it is likely that hydrocarbons are not a significant source of contamination at these two sites.

We did not observe any effects of UV radiation alone within our study, suggesting that the intensities of UV which were measured in the field are not high enough to singly harm amphibians. There was also no interaction between UV and semipermeable membrane device extracts from any site. This indicates that the compounds present in these waters are not broken down or photoactivated to be made less or more toxic, respectively.

Conclusions

Habitat decimation has been cited as being the major contributor of amphibian declines, but when declines are occurring in protected areas we are forced to consider other, more insidious, factors such as contamination. Often, habitats that appear pristine can be unfavorable for amphibians and other aquatic organisms. In our study, alterations in amphibian life history characteristics were evident within all three of the parks and wildlife refuges examined. Even if contaminants do not cause outright mortality, alterations in life history characteristics have the potential to alter population structure over time. For example, if postponing metamorphosis delays age at first reproduction, population growth rates

potentially decrease (STEARNS, 1992). If in a contaminated environment there were a shift toward later reproduction in an amphibian population due to delayed maturity, the demographic structure of the population may be altered, resulting in a gradual decline in size over time. Utilizing semipermeable membrane devices offers a way to sample aquatic habitats for contaminants in a non-destructive manner, and can be used to help assess the health of aquatic amphibian habitats.

RÉSUMÉ

Les declins des populations d'amphibiens ne sont pas limités aux habitats ouvertement dégradés, mais s'observent souvent dans des environnements relativement intacts tels que des parcs nationaux ou des réserves naturelles, forçant ainsi les biologistes à examiner des causes de déclin moins évidentes a priori, comme la présence de contaminants. L'objectif de notre étude était d'extraire, au moyen de membranes semi-perméables, des composés présents dans des habitats naturels d'amphibiens dans trois parcs nationaux ou réserves naturelles (Sequoia Kings Canyon National Park, Big Bend National Park et Kenai National Wildlife Refuge; deux sites étudiés pour chacun), et d'évaluer leur toxicité par des tests biologiques sur des larves. Les extraits ne causèrent pas de mortalité, et tous les effets observés furent sublétaux, modifiant des caractéristiques du développement. Dans les trois régions étudiées, les amphibiens élevés avec des extraits provenant d'au moins un des deux sites ont manifesté soit un allongement de la période larvaire soit une réduction de la masse à la métamorphose. Les extraits provenant de l'air et de l'eau d'un site du Sequoia Kings Canyon National Park allongèrent la période larvaire, ce qui est en accord avec les résultats d'études montrant des niveaux élevés de contaminants transportés par l'air dans de tels sites au sein du parc. Les radiations ultraviolettes, qui ont également eté soupçonnées d'avoir causé des déclins d'amphibiens, ne se sont pas avérées agir seules ou altérer la toxicité des extraits.

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